Vol. 4 No. 2, Dec. 2024

FUAM Journal of Pure and Applied Science

Available online at
www.fuamjpas.org.ng

An official Publication of
College of Science
Joseph Sarwuan Tarka University,
Makurdi.

FUAM Journal of Pure and Applied Science
© College of Science, Joseph Sarwuan Tarka University, Makurdi
https://fuamjpas.org.ng/
Vol.4 No. 2, December, 2024

Evaluation of intestinal microbial counts and fecal mineral contents of broiler chickens fed varying dietary levels of activated charcoal

S.N*[1]. Okey, C.C[1]. Ogbu, S.C[2]. Akomas, I. C[3]. Okoli, C.A. N[4]. Akpan, C.R[1]. Unigwe and O.N[5]. Okey

[1]Department of Veterinary Biochemistry and Animal Production, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Nigeria
[2]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Nigeria
[3]Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria
[4]Department of Veterinary Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Nigeria
[5]God's Gift Veterinary Services, Uyo, Akwa Ibom State, Nigeria
*Correspondence E-mail: nnamuokey@gmail.com

Received: 24/05/2024 **Accepted**: 14/06/2024 **Published online**: 17/06/2024

Abstract
The resistance associated with the use of antibiotics has necessitated the dietary supplementation of adsorbents such as activated charcoal (AC) in eliminating pathogens in poultry. One hundred and twenty unsexed day old arbor acre broilers were used. The birds were distributed into four treatment groups (G1-G4) of thirty birds per treatment in a completely randomized design. G1 was control while G2-G4 was fed diet which contained 0.5g/kg, 1.0g/kg and 1.5g/kg of AC respectively. On 7, 28 and 42 days of age, fecal samples were pooled over a 24-hour period from three birds randomly selected from each treatment group for microbial and faecal mineral analyses using standard laboratory techniques. Data collected were analyzed statistically using Analysis of Variance (ANOVA) and significant differences were accepted at 0.05. Total *lactobacillus* counts were significantly ($p < 0.05$) higher in the supplemented groups than in the control group on 7 and 28 days of age while total *Salmonella* and *E. coli* counts were significantly ($p < 0.05$) reduced in the supplemented groups on 28 and 42 days of age. It is concluded that AC increased *Lactobacillus* counts and reduced *E. coli* and *Salmonella* counts. Activated charcoal could therefore be used to modulate the gastro-intestinal microbiome of broiler chickens.
Keywords: Feed additive, broiler chickens, intestinal microbiome, microbial counts, fecal mineral content.

Introduction

Activated charcoal (AC) also known as biochar is a solid, porous, tasteless and black carbonaceous material [1] produced from a variety of carbon containing materials including agricultural wastes. Numerous studies have described the absorbance properties and potential clinical benefits of activated charcoal [2]. It is effective in the elimination of mycotoxins, such as aflatoxins as well as pesticide residues that occasionally contaminate feed ingredients [3]. The use of activated charcoal in animal farming reduces the harmful effects of ammonia and has the potential of reducing greenhouse gases such as methane and nitrous oxide [4]. Hitherto, AC was only used as universal poison antidote to tackle indigestion and for emergency treatment of poising in animals [5]. This is because of its adsorption capacity for toxins such as mycotoxins, plant toxins, and pesticides, as well as for pathogens. It is also known for its enteral dialysis property due to its ability to remove toxins from the blood plasma through interaction with the permeability properties of the intestine [6].

Activated charcoal has a strong adsorption capacity for gram-negative bacteria with high metabolic activity such as *E. coli* and *Salmonella* [7]. According to [8], mixture of feeds with 1% and 1.5% bamboo activated charcoal and bamboo vinegar, respectively slightly and significantly reduced the level of *E. coli and Salmonella* in chicken excreta. A patented biochar-wood vinegar product, Nekka-Rich® (Miyazaki-Midori Pharmaceuticals Inc. Miyazaki, Japan) showed a highly significant reduction of *Salmonella* in chicken droppings and increased the proliferation of beneficial intestinal flora (*Enterococcus faecum*) bacteria [9]. According to the author, the effect of AC on the suppression of the pathogenic bacterial species was of the same order of magnitude as that of antibiotics. In vitro studies revealed that AC as well as clay can effectively immobilize cattle rotavirus and coronavirus at rates of 79-99.9% [10]. This binding was made possible

since the diameter of the viral particles were larger than the pore diameters of clay and most pores of AC coupled with the viral surface proteins. Chicken feed was supplemented with 1% rice husk AC and it resulted in reduction of plasma triglycerides, total coliform bacteria in litter and *E.. coli in* faeces with no impact on live weight gain, feed consumption and feed conversion ratio [11]. More so, [12] reported that feeding AC to laying hens at 5% inclusion in diet reduced intestinal inflammation and improved gut development by increasing the villus height and area in the duodenum. Activated charcoal had been reported to contain many compounds including phenolics, alkanes, alcohol, aldehydes and organic acids especially acetic acids. The acetic acid component may contribute in shifting the balance of intestinal microflora in favour of beneficial microbiota and hence enhanced intestinal functions and metabolism [13]. It was observed that increased intestinal villus height enhances digestion and absorption capacity of the small intestine while increased population of beneficial bacteria enhances the supply of nutrients and promote vascularization and development of the intestine [14]. The author also observed shorter intestinal villi, fewer absorptive and secretory cells following increased counts of pathogenic bacteria in the chicken intestine. Activated charcoal addition to diet of chickens can help recover intestinal integrity, improve gut health and increase nutrient availability and absorption [15, 16].

Poultry faeces is a mixture of unabsorbed nutrients, undigested and non-digestible feed materials, uric acid, cells of the digestive mucosa, and several minerals. On the average, broiler chicken produces 120-140 grams of faeces per day [17]. Poultry faeces contain 13 of the essential minerals that are used by plants which include nitrogen, phosphorus, potassium, calcium, magnesium, Sulphur, manganese, copper, zinc, chloride, boron, iron and molybdenum [18]. Knowledge of the mineral content of poultry faeces enhances understanding of the state of mineral nutrition as well as reveals disturbances in mineral metabolism. In view of the threat to global health by antimicrobial resistance, it becomes imperative to source alternative feed additives for poultry for purposes of disease prevention and control, growth promotion, and enhanced feed efficiency. The present study was hence undertaken to evaluate the effect of dietary AC on intestinal microbial count and faecal mineral content of broiler chickens.

Materials and Methods

Ethical Approval

Ethical approval was obtained from College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike Ethical Committee on the care, use and management of animals in experiments with code MOUAU/CVM/REC/2019/112.

Location of the Study

The study was carried out at the Teaching and Research Farm of Michael Okpara University of Agriculture Umudike, Abia State. Umudike is located within the South East agro-ecological zone of Nigeria with geographical coordinates of 5.4801◦ N and 7.5437◦ E.

Activated charcoal preparation

The AC employed in the present study was prepared by thermal decomposition or carbonization of the precursors followed by steam activation following the procedure outlined by [19]. Details of the carbonization and activation processes had been presented by [20]. Briefly, a blend of palm kernel shell, pig dung, and palm fruit fiber (4:3:3 by weight) was sun-dried to constant weight and then thermally carbonized in earthen pot under limited atmospheric oxygen. At complete combustion indicated by cessation of smoke emission from material, physical activation was achieved by rapid introduction of water into the red hot char with the pot covered immediately. On cooling, the activated charcoal was removed from the pot, sun-dried to constant weight and ground to powder using a blender. The material was stored in airtight container until used.

Experimental animals and study design

One hundred and twenty unsexed day old arbor acre broilers were used. They were distributed into four treatment groups (G1-G4) with each comprising of 30 birds in a completely randomized design. They were maintained ad-libitum on a starter and finisher diet for 3 weeks each with G1 as control. G2-G4 was fed diet which contained 0.5g/kg, 1.0g/kg and 1.5g/kg of AC respectively. On the days 7, 28 and 42 of age, three birds were randomly selected from each treatment and housed separately in metal cages lined with polythene sheets and used to collect excreta every 4 hours for a 24-hour period. Collected faeces were held in plastic bags and refrigerated at 5◦C immediately after collection to minimize microbial degradation. Faeces from each treatment were thoroughly mixed and used for microbiological and faecal mineral analysis following standard laboratory techniques.

Isolation, Identification and Colony count of Microbial Organisms in Intestinal Digesta

1. *Escherichia coli*
2. *Lactobacillus*
3. *Salmonella*

The microbial (*E. coli*, *Lactobacillus* and *Salmonella* counts) of the digesta was determined using standard bacteriological techniques [21]. The counting of the total bacteria was carried out using a colony counter (Model: GMP, India) and rendered in colony forming units (CFU) per gram digesta and calculated as: number of colonies counted x dilution factor.

Determination of Mineral Composition of Faecal Sample

The mineral contents namely sodium (Na), calcium (Ca), potassium (K), phosphorus, magnesium (Mg), zinc (Zn), iron (Fe) and copper (Cu) of broiler faeces were measured

using the Atomic Absorption Spectrophotometer method (Buck Scientific, 205, India) following the procedure of spectrophotometry. The procedure was based on the principle that metallic elements in a ground form absorb light of the same wavelength which they emit when excited, with the amount of radiation absorbed being directly proportional to the concentration of the element present. 2g of each fecal sample was placed in a Kjeldahl flask and 20ml of concentrated nitric acid (HNO3) added to it. The sample was pre-digested by heating gently for 20-40 minutes with addition of more acid until a clear digest was obtained. The flask was allowed to cool and the contents filtered into a 50ml volumetric flask through 150 nm diameter filter paper followed by addition of distilled water added to make up the volume. The resultant solution was analyzed for mineral elements.

Results and Discussion

Table I, presents the microbial counts of the experimental birds at 7, 28 and 42 days of age.

Table 1: Total intestinal *E. coli*, *Lactobacillus* and *Salmonella* counts (CFU/g) x 10^3 of broiler chickens fed varying levels dietary of activated charcoal

Organism	Age (days)	Experimental groups				
		G1	G2	G3	G4	p-value
E. coli	7	27.10±4.95	25.80±4.56	25.20±7.04	24.70±1.48	0.978
	28	11.90±3.03^{a}	6.83±0.25^{b}	4.73±0.61^{b}	4.00±0.69^{b}	0.001
	42	11.37±2.67^{a}	6.93±0.49^{b}	5.33±0.87^{b}	5.10±0.56^{b}	0.002
Lactobacillus	7	10.67±0.50^{b}	16.23±2.68^{ab}	17.83±1.15^{a}	14.26±2.29^{ab}	0.015
	28	5.10±1.05^{b}	8.00±0.79^{a}	6.73±1.01^{ab}	9.10±0.52^{a}	0.003
	42	6.07±0.93^{b}	8.37±0.67^{ab}	6.63±1.83^{b}	9.47±1.25^{a}	0.035
Salmonella	7	23.77±11.33	19.13±8.46	19.73±8.40	33.43±14.54	0.408
	28	12.67±2.06^{a}	10.00±1.32^{ab}	7.33±0.15^{b}	4.27±0.49^{c}	0.000
	42	12.93±2.50^{a}	9.60±1.15^{ab}	6.80±1.55^{bc}	4.63±3.23^{c}	0.001

Results are mean ± SD; a, b, c: means on the same row with different superscripts are significantly different ($P < 0.05$).

The observed significantly lower intestinal pathogenic bacteria (*E. coli and Salmonella*) colony counts and higher beneficial intestinal bacteria (*Lactobacillus*) counts in the supplemented groups was in conformity with the report of [22] that AC given orally reduced intestinal *Salmonella enteritidis* and minimized the removal of beneficial bacteria flora (*Enterococcus faecium and Lactobacillus*) from the intestinal tract. Similarly, [23] discovered supplementing noiler chicken feed and litter with 1% rice husk AC reduced the total coliform counts in the litter and *E. coli* in faeces. A commercially available AC (Nekka-Rich®) was fed as prebiotic to Leghorn chickens by [24] who observed significant reduction in *Salmonella* recovered from the large intestine and faeces. [25] also reported that inclusion of bamboo charcoal in the diet of fattening pigs minimized the shedding of *Salmonella* in faeces while [26] indicated that intake of AC removes toxic heavy metals and pathogenic bacteria from the blood.

In poultry, the intestinal microbiome form synergistic relationship with the host gastrointestinal (GIT) environment and significantly impact the uptake and utilization of nutrients. They are also essential for the development of the gut epithelium, and mucosal immunity; and they confer many other benefits to the physiology of the gut. By regulating the immune cells, the intestinal microbiota enhances mucosal barrier function enabling the host to mount a robust immune response against invading pathogens thereby maintaining immune homeostasis [27, 28]. Disturbance of the gut microbial balance can lead to dysbiosis and or enteritis which negatively impacts host metabolism, and energy utilization leading to increases in pathogenic bacteria load. The observed limiting effect of AC on intestinal population of *E. coli* and *Salmonella* species and the enhanced population of *Lactobacillus* in the present study, indicate that adding AC to diet of broiler chickens can help modulate intestinal microbial community to enhance intestinal integrity, improve gut health, increase nutrient availability, nutrient absorption, and utilization [29]. The faecal sample content mineral characteristics of broiler chickens fed the experimental diets is presented in Table 2.

Table 2: Faecal mineral characteristics of broiler chickens fed varying dietary levels of activated charcoal

Group	Wk	Na	Ca	K	P	Mg	Zn	Fe	Cu
G_1	3	1.54	0.68	1.35	1.45	0.40	160	950	25
G_2		1.64	0.70	1.40	1.42	0.40	160	956	25
G_3		1.64	0.68	1.38	1.45	0.40	158	950	25
G_4		1.70	0.68	1.38	1.43	0.42	156	950	25
G_1	6	1.26	0.65	1.38	1.48	0.42	158	950	25
G_2		1.26	0.62	1.38	1.45	0.45	160	955	25
G_3		1.12	0.65	1.36	1.48	0.42	155	955	26
G_4		1.46	0.65	1.38	1.48	0.40	155	950	25

Phosphorus, Potassium, Calcium and Magnesium obtained in this study were below the range of 2.46-2.82, 2.02-2.32, 4.52-8.15 and 0.52-0.73% respectively as reported by Department of Sustainable Organic Agriculture, Tamil Nadu Agricultural University, Coimbatore in 2009. Large variations in the level of minerals and nitrogen were observed in other reported results. The magnesium content was higher than 0.28% reported at Clemson University in 2001. The fecal mineral content of broiler chickens is highly variable and depends on various factors such as feed given to the birds, its moisture content, age of the birds, production and feeding system, feed consumption and composition. Environmental factors like temperature and ventilation can also affect the fecal mineral content of broilers.

Conclusion

The observed significantly lower *E. coli* and *Salmonella* counts and higher *Lactobacillus* counts in AC supplemented groups indicate that AC supplementation in diet reduced intestinal tract colonization by pathogenic micro-organisms while enhancing colonization by beneficial microflora. The observed faecal mineral contents of AC supplemented groups were essentially similar to those of the control group. This indicates that AC supplementation in diet did not markedly increase mineral excretion in faeces of the supplemented groups. Therefore, AC supplementation in diet of broiler chickens could be used to modulate intestinal microflora to enhance health and productivity and could serve as an alternative to antibiotics feed additives in broiler production.

References

[1] AAFCO. 2012. Official publication. Association of American Feed Control Officials. USA.

[2] Maklad, A.I., Ashraf, M.E., Enas, I. E. and Mennat-Allah, A. M. E. 2012. **Pediatric poisoning in Egypt.** *Journal of Poultry and Applied Pharmaceutical Science,* 2: 1-6.

[3] Bhatti, S.A., Khan M.Z., Hassan, Z.U., Saleemi, M.K., Saqib, M., Khatoon, A. and Akhter, M. 2018. **Comparative efficacy of bentonite clay, activated charcoal and Trichosporonmycotoxinivorans in regulating feed-to-tissue transfer of mycotoxins**. *Journal of Food and Agriculture* 3: 884 – 890.

[4] Sashikala, M., Pillai, G. P., Xinguang, R. and Stuetz, M. 2012. **Odour abatement of poultry litter using odour control products.** *A publication of Italian Association of Chemical Engineering*, 30. Online: www.acidic.it.cet.

[5] Hagemann, N., Spokas, K. Schmidt, H.P., Kagi, R., Bohler, M.A. and Bucheli, T. 2018. **Activated Carbon biochar and charcoal linkages and synergies across pyrogenic carbon's ABCs**. *Water*, 2:182.

[6] Schmidt, H.P., Hageman, N., Drapper, K. and Kammann, C. 2019. **The use of biochar in animal feeding**. *Peer Journal 7: e7373.*

[7] Kim, K.S., Kim, Y.H., Park, J.C., Yun, W., Jang, K.I., Yoo, D.I., Lee, D.H., Kim, B.G. and Cho, J.H. 2017. **Effect of organic medicinal charcoal supplementation in finishing pig diets**. *Korean Journal of Agricultural Science*, 44: 50-59.

[8] Watarai, S. and Tana, S. 2005. **Eliminating the carriage of *Salmonella enterica serovar enteritidis* in domestic fowls by feeding activated charcoal from bark containing wood vinegar liquid** (Nekka-rich). *Poultry Science*, 84: 515-521.

[9] Schmidt, H. P., Hagemann, N., Draper, K., & Kammann, C. (2019). **The use of biochar in animal feeding**. *PeerJ*, 7, e7373..

[10] Hien, N. N., Dung, N. N. X, Manh, L. H. and Le Minh, B. T. 2018. **Effect of biochar inclusion in feed and chicken litter on growth performance, plasma lipids and fecal bacteria count of Noi lai chicken**.

Journal of Livestock Research for Rural Development, 7: 131.

[11] Hien, N. N., Dung, N. N. X, Manh, L. H. and Le Minh, B. T. 2018. **Effect of biochar inclusion in feed and chicken litter on growth performance, plasma lipids and fecal bacteria count of Noi lai chicken**. *Journal of Livestock Research for Rural Development*, 7: 131.

[12] Rattanawut, J., Areerat, A and Wigunda, R. 2021. **Supplemental effect of bamboo charcoal and bamboo vinegar, alone or in combination, on laying performance, egg quality, intestinal bacteria populations and alteration of intestinal villi**. *Italian Journal of Animal Science*, 1: 2211-2219.

[13] Lin, H.C., Murase, Y., Shilah, T.C., Hwang, G.S., Chen, P.K. and Wu, W.L. 2008. **Application of moso bamboo vinegar with different collection temperatures to evaluate fungi resistance of moso bamboo materials.** *Journal of Faculty of Agriculture Kyushu University*, 53: 107-113.

[14] Choct, M. 2009. **Managing gut health through nutrition**. *British Poultry Science,* 50: 9 - 15.

[15] Awad, W.A., Ghareeb, K., Abdel-Raheem, S. and Bohm, J. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science,* 88: 49 - 55.

[16] Abdelquader, A., Al-Fataftah, A.R. and Das, G. 2013. **Effects of dietary *Bacillus subtillis* and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production**. *Animal Feed Science and Technology*, 179: 103 - 111. [26]

[17] TNAU. 2009. **Organic farming. Department of Sustainable Organic Agriculture, Tamil Nadu Agricultural University,** Coimbatore-641003.http://agritech.tnauac.in//org_farm/orgfarm.

[18] Chastain, J., Camberato, J.J. and Albrecht, J.E. 2001. **Nutrient content of livestock and poultry at Clemson University**. www.clemson.edu/extention/camm/manuals/poultrypch3b_00.pdt.

[19] Gunamartha, I.M. and Widana, G.A.B. 2018. Characterization of the potential of biochar from cow and pig manure for genecology application. *Conference Series: Earth Environmental Science*, 131: 12-55.

[20] Okey, S.N., Ogbu, C.C., Okoli, I.C., Okey, O.N., Akpan, C.A.N., Afolabi, K.D. and Udo, U.J. 2022. **Effect of dietary inclusion of agricultural waste-derived activated charcoal on haematological and serum biochemical indices of layer chickens.** *Asian Journal of Research in Animal and Veterinary Sciences*, 4: 1-11.

[21] Cruickshank, R., Duguid, J.P., Marmion, B.P and Swain, R.H.A. 1989. **Medical Microbiology, 12th edition, Churchhill Living Stone, Edinburgh, UK**.

[22] Yamauchi, K., Ruttanavut, J. and Takenoyama, S. 2010. **Effect of dietary bamboo charcoal powder including vinegar liquid on chicken performance and histological alterations of intestine**. *Journal of Animal Feed Science,* 19: 257-268.

[23] Kogut, M. H. 2013. **The gut microbiota and host innate immunity: regulators of host metabolism and metabolic diseases in poultry**. *Journal of Applied Poultry Research,* 22: 637-646.

[24] Mabbet, T. 2005. **Integrated management of mycotoxins**. *Poultry International*, 8: 10-14.

[25] Chu, G.M., Jung, C.K., Kim, H.Y., Ha, J.H., Kim, J.H., Jung, M.S., Lee, S.J., Song, Y., Ibrahim, R.I.H., Cho, J.H., Lee, S.S. and Song, Y.M. 2013. **Effect of bamboo charcoal and bamboo vinegar as antibiotics alternatives on growth performance, immune responses and fecal microflora population in fattening pigs**. *Animal Science Journal*, 84: 113-120.

[26] Roosdiana, A., Vidiastuti, D and Herenda, H. 2019. **The preventive effect of activated charcoal on HDL levels and aorta histopathological profiles in hypercholesterol rat models.** *Journal of Physics*: Conference Series, 1374: 012029.

[27] Brisbin, J. T., Gong, J. and Sharif, S. 2008. **Interactions between commensal bacteria and the gut-associated immune system of the chicken**. *Animal Health Research Review*, 9: 101-110.

[28] Forder, R. E., Naltrass, G. S., Geier, M. S., Hughes, R. J. and Hynd, P. I. 2012. **Quantitative analyses of genes associated with mucin synthesis of broiler chickens with induced necrotic enteritis**. *Poultry Science*, 91: 1335-1341.

[29] Sommer, F. and Backhead, F. 2013. **The gut microbiota masters of host development and physiology.** *Nat. Review of Microbiology*, 11: 227-238.

Cite this article

Okey S.N., Ogbu C.C., Akomas S.C., Okoli I.C., Akpan C.A.N., Unigwe C.R. and Okey O.N. (2024). Evaluation of intestinal microbial counts and fecal mineral contents of broiler chickens fed varying dietary levels of activated charcoal.
FUAM Journal of Pure and Applied Science, **4**(**2**):48-52